tions in determining the fate of the transplant, particularly in the case of organ transplants in which the immune system of the recipient reacts with donor blood vessels (Fig. 1*b*). First, cytokines contribute to the differentiation of helper T cells and B cells in lymphoid organs. Effector cells such as Th1 cells or cytotoxic T cells develop first, followed by immunoregulatory cells that control or constrain immune responses. Second, cytokines and other inflammatory mediators act on blood vessels and parenchymal cells of the transplanted tissue, initially inducing acute injury. Later, cytokines may induce a compensatory response in the graft, leading to resistance to tissue injury. This effect is known as accommodation[6].

Elucidation of the function of IFN-γ may be particularly instructive in determining mechanisms of graft rejection and resistance to injury. In the acute setting (a period of a few days), IFN-γ acts on antigen-presenting cells in lymphoid organs to promote the acute cellular immune responses that cause cellular rejection. IFN-γ released by effector cells in the transplanted tissue may enhance the susceptibility of blood vessels to injury by cytolytic T cells, complement and other inflammatory mediators. If the transplanted tissue is able to survive this stage of the immune response, then over a longer period of time IFN-γ may contribute to the generation of immunoregulatory T cells that constrain or inhibit cellular immunity. Consistent with this, Konieczny *et al.* found that IFN-γ promotes the long-term survival of allografts in recipients in which acute destruction of the transplant is prevented[7]. IFN-γ may also induce a compensatory response by the vasculature of the graft that helps the graft resist injury, as toxic substances can eventually induce resistance to toxic injury[8].

The ability of IFN-γ to induce nitric oxide synthetase may be of particular importance in determining graft survival or rejection[9]. Nitric oxide, produced by nitric oxide synthetase, might cause acute oxidant-mediated injury but over time induce radical scavengers and heme oxygenase that confer resistance oxidant injury. Nitric oxide may also protect the graft by preventing the constriction of blood vessels, an event we believe is seminal in the pathogenesis of AVR. Xenotransplants may be especially susceptible to AVR because the interaction of IFN-γ with its receptor exhibits some degree of species specificity. In such cases, IFN-γ produced by recipient cells is not recognized by blood vessels of the graft, and resistance to injury might not ensue.

The findings of Wang *et al.* have implications beyond xenotransplantation. If two strains of mice have such profound differences in susceptibility to AVR, humans might have similar differences. Polymorphisms of the IFN-γ receptor have already been linked to susceptibility to infection and other diseases. There is also every likelihood that manifestations of immune or inflammatory responses may in some cases reflect less the peculiar mix of cytokines secreted by recipient leukocytes in areas of inflammation and injury than the way in which cells of the graft are poised to respond to those cytokines. The effect of cytokines may be determined by the properties of the responding cells.

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# Caspase 8: The killer you can't live without

**The proto-oncogene *MYCN*, a regulator of cell proliferation and cell death, is amplified in aggressive neuroblastomas, whereas expression of the apoptotic effector caspase 8 is suppressed. Understanding why this nervous system tumor should choose to up or down regulate these particular apoptotic factors may provide important information about oncogenesis and indicate new strategies for the treatment of neuroblastoma (pages 529–535).**

PHILIPPE JUIN & GERARD EVAN

THE COUPLING OF cell proliferation and cell suicide is thought to be an important cellular mechanism for the prevention of oncogenesis[1], and it is believed that growth-deregulating mutations can lead to neoplasia only when apoptosis has been suppressed. It is therefore of great clinical importance to determine how apoptosis becomes suppressed in cancer, as repair of a cancer cell's defective apoptotic machinery offers the promise of effective and specific anti-cancer therapy. In this issue of *Nature Medicine*, Teitz *et al.* have investigated mechanisms of apoptotic gene inactivation in neuroblastomas, tumors of the peripheral sympathetic nervous system that most commonly occur in children[2].

Teitz *et al.* report that a large proportion of aggressive neuroblastoma cells, which are particularly resistant to induction of apoptosis, suppress expression of caspase 8, an important component of the apoptotic machinery[3]. Caspases are a family of cysteine aspartyl proteases that are activated in proteolytic cascades during cell death[4]. The process of cell dissolution we call apoptosis is implemented by the abundant effector caspases, such as caspase 3, which act as intracellular 'chain saws' to cleave essential intracellular substrates. These effector caspases are activated by apical or initiator caspases, which autoactivate when induced to oligomerize through their extensive 'pro-domains' in response to a variety of pro-apoptotic signals (Fig. 1). For example, apical cas-

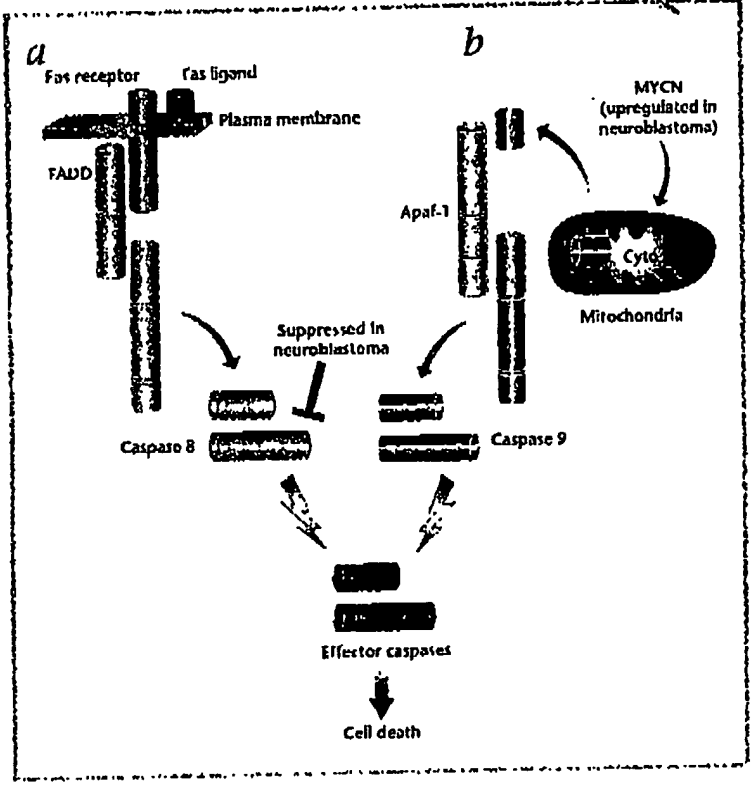


Fig. 1 Activation of the apical caspases 8 and 9. *a*, Ligation of the CD95/Fas receptor triggers recruitment of the precursor form of caspase 8 to a death-inducing complex, through the adapter protein FADD, and its consequent auto-activation. *b*, Various signals trigger release of mitochondrial holocytochrome (Cyto c) into the cytosol. Formation of a ternary complex comprising Cyto c, Apaf-1 and the precursor form of caspase 9 induces auto-activation of caspase 9. Active forms of caspase 8 and 9 initiate a cascade of effector caspases. The caspase 9 pathway (8) is directly activated by MYC proteins (MYCN) as a result of their ability to induce Cyto c release by an unknown mechanism. Nonetheless, the caspase 9 and caspase 8 pathways cooperate for efficient induction of apoptosis. Silencing of caspase 8 in neuroblastoma confers resistance to MYC-induced apoptosis.

pase 9 is activated when holocytochrome c is released from mitochondria and orchestrates the assembly of caspase 9 and its activator, apoptotic protease-activating factor (Apaf)-1. In contrast, caspase 8 is activated after ligation of specific transmembrane death receptors, most notably the prototypic death receptor CD95/Fas/Apo1, which has important functions in immune homeostasis and surveillance. The diversity of mechanisms involved in the regulation and implementation of apoptosis is indicative of a rich variety of targets for its inhibition during oncogenesis. For example, some tumor cells over express the anti-apoptotic Bcl-2 proteins, which restrict release of cytochrome c and consequent activation of caspase 9; in other examples, activation of the FLIP proteins or NF-κB suppresses death receptor signaling, and inactivation of p53 can incapacitate activation of apoptosis after genotoxic damage.

The MYC oncoproteins function as both promoters of cell proliferation and as activators of apoptosis[1,3]. *MYCN* is a functional homolog of the prototypic *MYC* oncogene *C-MYC*, whose amplification in neuroblastomas is an adverse prognostic factor. However, overexpression of *MYCN* has also been shown to sensitize neuroblastoma cells to apoptotic signals[4]. Consequently, there has been great interest in determining how apoptosis induced by MYCN is forestalled despite its overexpression. The findings of Teitz *et al.* indicate that loss of caspase 8 expression provides an essential mechanism responsible for thwarting *MYCN*-induced cell suicide[2].

The authors examined 18 human neuroblastoma cell lines and found that 13 lacked expression of caspase 8 RNA and protein[2]. However, only one line had a deletion of the gene for caspase 8 (*CASP8*), and there was no evidence of mutations within *CASP8* coding regions. Instead, expression seemed to be silenced through methylation of *CASP8* DNA regulatory sequences. Similarly, there was also silencing of *CASP8* in a proportion of cryopreserved neuroblastoma samples and, most provocatively, methylation and silencing of *CASP8* correlated almost perfectly with amplification of MYCN in both the neuroblastoma cell lines and patient samples. Such correlation indicates that neuroblastoma cells with increased expression of *MYNC* are subject to strong selective pressure to lose caspase 8 function.

As might be predicted, loss of expression of caspase 8 confers protection against apoptosis. Neuroblastoma cell lines lacking caspase 8 show considerable resistance to apoptosis induced by ligation of the death receptors Fas, tumor necrosis factor receptor p55 or death receptor 3, as well as by FADD, the intracellular death receptor adaptor protein[2]. This resistance is reversed with re-introduction of caspase 8 into deficient neuroblastoma cells, and is consistent with the known obligate function for caspase 8 in death-receptor-induced apoptosis, as shown in embryonic fibroblasts deficient in caspase 8. Presumably, resistance to death-receptor-induced apoptosis could be advantageous to some tumor cells, as it would render them more resistant to surveillance by cytotoxic T cells. However, neuroblastoma cells deficient in caspase 8 also show resistance to the genotoxic drug doxorubycin[2], consonant with studies indicating that some DNA-damaging agents promote cell death by inducing surface expression Fas ligand, thereby triggering autocrine activation of CD95/Fas and consequent activation of caspase 8 (ref. 7). Drug resistance has obvious selective advantages for tumor cells.

It is less clear why loss of expression of caspase 8 should be associated with amplification of MYCN, since caspase 8 has not been shown to be directly involved in MYC-induced apoptosis. Caspase 9, however, is directly activated by MYC-induced release of cytochrome c from the mitochondria into the cytosol[8], and inactivation of caspase 9 enhances MYC-induced transformation[9]. Therefore, it will be interesting to learn why expression of caspase 9 is apparently unaffected in neuroblastoma cells[2].

Nonetheless, there is evidence of synergy, even interdependence, between caspase 9 and caspase 8 signaling pathways. Expression of c-myc sensitizes cells to CD95/Fas-induced apoptosis, and C-MYC or Fas signaling, which are

independently incapable of inducing cell death, trigger apoptosis when they are both activated in the same cell. Indeed, Fas signaling is required for induction of apoptosis by C-MYC in serum-deprived rodent fibroblasts[10]. It seems that cytosolic holocytochrome c is not sufficient to activate apoptosis but requires a secondary signal from the CD95/Fas receptor to trigger cell death[8]. The mechanism for synergy between CD95 and cytochrome c is unknown at present, but it may well require active caspase 8 to amplify a caspase 9 cascade[11].

Other mechanisms for the suppression of MYC-induced apoptosis have been seen in cancer cells, including up-regulation of the anti-apoptotic factor Bcl-2/Bcl-$x_L$, possibly loss of Apaf-1, and direct inhibition or ablation of the CD95 ligand or receptor. Why, then, does methylation and silencing of *CASP8* seem to be the favored mechanism for suppressing apoptosis in neuroblastomas with amplified *MYCN*? Is their some peculiar idiosyncrasy of neuroblastoma development that necessitates ablation of caspase 8, or is it merely that loss of caspase 8 is more widespread in human cancers and has been overlooked so far? Does the silencing of caspase 8 occur instead of, or in addition to, other anti-apoptotic mutations? Why is caspase 9, an established effector of MYC-induced apoptosis, not silenced in any of the neuroblastomas examined?[2] And finally, would re-activation of caspase 8 constitute an effective therapeutic strategy for the treatment of neuroblastomas that have amplified *NMYC*? These are important, and now answerable, questions with direct implications for the treatment of this tragic childhood cancer.

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# Chemotherapeutic drugs—more really is not better

**Recent insights into the molecular mechanisms that regulate the process of metastasis and the complex interactions between metastatic cells and host factors have provided a biological foundation for the design of more effective therapies.**

ISAIAH J. FIDLER & LEE M. ELLIS

Most deaths from cancer are due to metastases that are resistant to conventional therapies. The search for the mechanisms that regulate the pattern of metastasis began over a century ago. In 1889, Paget investigated the distribution of metastases by analyzing a large number of autopsy records of women with breast cancer. Noting the nonrandom pattern of visceral metastases, he concluded that the process was due not to chance but rather to the specific affinity of certain tumor cells for certain organs. Thus, metastases resulted only when the tumor cells and organ environment were compatible[1].

A current version of this hypothesis consists of three principles. First, neoplasms are biologically heterogeneous and contain subpopulations of cells with different angiogenic, invasive and metastatic properties; their response to therapeutic agents is likewise heterogeneous[2]. Second, the process of metastasis is selective for cells that succeed in the steps of induction of angiogenesis, invasion, embolization, transport in the circulation, arrest in a distant capillary bed and extravasation into and multiplication within the organ parenchyma[2]. Although some of the steps (Fig. 1) contain stochastic elements, as a whole, metastasis favors the survival and growth of a few subpopulations of cells that preexist within the parent neoplasm[2,3]. The third and perhaps most relevant principle to cancer therapy design is that the outcome of metastasis depends on multiple interactions of metastatic cells with homeostatic mechanisms[4]. Therapy of metastasis, therefore, could be targeted not only against tumor cells but also against the homeostatic factors that are favorable to the growth and survival of metastatic cells. Two reports, recently published in the 1 April issue of *Cancer Research* (by Browder *et al.*[5]) and the 15 April issue *The Journal of Clinical Investigation* (by Klement *et al.*[6]), support this theory, suggesting drugs designed to kill tumor cells also act on the tumor endothelium to inhibit neoplastic angiogenesis.

Induction of angiogenesis is an essential step in the continuous growth of primary neoplasms and in the process of metastasis[7]. Angiogenesis is a multi-step pathway involving local degradation of the basement membrane surrounding capillaries followed by invasion of the stroma by the underlying endothelial cells, which proliferate and develop into mature blood vessels[8]. Therapeutic targeting of neoplastic angiogenesis was first proposed by Folkman in 1971 (ref. 9). Recent improvements in our understanding of the cellular and molecular basis of this process[7,10] have stimulated a plethora of reports detailing the rational design of different specific approaches to inhibiting angiogenesis. Many of these are now undergoing clinical trials.

Suppression of angiogenesis has also been achieved by cancer chemotherapy originally designed to directly destroy cancer cells. For example, administration of high doses of doxorubicin led to regression of doxorubicin-resistant